

**DATA EVALUATION RECORD
CHRONIC TOXICITY TEST WITH THE HONEYBEE
Non-Guideline Chronic Feeding Study**

1. **CHEMICAL**: Mesotrione

PC Code: 122990

2. **TEST MATERIAL**: Mesotrione technical

Purity (analyzed): 84.6% w/w

3. **CITATION**

Author: Wendling, K.

Title: Mesotrione –Honey Bee (*Apis mellifera* L.) Chronic Oral Toxicity Test 10 Day Feeding Test in the Laboratory

Study Completion Date: December 10, 2018

Laboratory: Eurofins Agrosience Services EcoChem GmbH

Sponsors: Syngenta Ltd

Laboratory Report ID: S18-03658

DP Barcode: 450340

MRID No.: 50743201

4. **REVIEWED BY**: Holly E. Dimig, Junior Staff Scientist, CDM/CSS-Dynamac JV

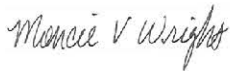
Signature:



Date: 3/26/2019

APPROVED BY: Moncie V. Wright, Ph.D., Environmental Scientist, CDM/CSS-Dynamac JV

Signature:



Date: 4/3/2019

5. **REVIEWED BY**: Michael Lowit, Ph.D., Senior Scientist, EPA/OCSP/OPP/EFED

Signature:



Date: 10-2-2020

6. STUDY PARAMETERS

Age of Test Organisms at Test Initiation: Young adult worker bees (1 to 2 days old)

Exposure Duration: 10 days

7. CONCLUSIONS

The honeybee, *Apis mellifera* L., was exposed to mesotrione technical for 10 days in a feeding study at the nominal concentrations and doses in the table below. The mean-measured diet concentrations were calculated by the study author. The reviewer used the reported mean % recoveries to calculate the measured actual intake dietary doses. All values are reported in the table below.

Nominal Concentration (mg ai/kg diet)	Mean-Measured Concentration (mg ai/kg diet)	Nominal Actual Intake Dietary Dose (µg ai/bee/day)	Measured Actual Intake Dietary Dose (µg ai/bee/day)
62.5	67.7	1.41	1.52
125	115	2.73	2.5
250	224	5.92	5.3
500	443	13.30	12
1000	880	21.85	19

After 10 days, mortality was 5% in both the negative and solvent controls compared to mortality ranging from 0 to 15% in the treatment groups. Mean food consumption over the course of the study was 29.6 and 22.0 mg/bee/day in the negative and solvent controls, respectively, compared to mean consumption of 22.6, 21.9, 23.7, 26.6, and 21.8 mg/bee/day in the measured actual intake dietary dose groups of 1.52, 2.5, 5.3, 12, and 19 µg ai/bee/day, respectively. No behavioral abnormalities were observed in any of the control or treatment groups throughout the 10-day test period.

The 10-day NOAEC and LC₅₀/IC₅₀ were 880 and >880 mg ai/kg diet, respectively (corresponding to a NOAEL and LD₅₀/ID₅₀ of 19 and >19 µg ai/bee/day, respectively).

	Mortality	Food Consumption
Dietary Concentration (mg ai/kg diet)	LC ₅₀ >880 95% C.I.: N/A Slope: N/A NOAEC ≥ 880 LOAEC >880	IC ₅₀ >880 95% C.I.: N/A Slope: N/A NOAEC ≥ 880 LOAEC >880
Dietary Dose (µg ai/bee/day)	LD ₅₀ >19 95% C.I.: N/A Slope: N/A NOAEL ≥ 19 LOAEL > 19	ID ₅₀ >19 95% C.I.: N/A Slope: N/A NOAEL ≥ 19 LOAEL > 19

8. ADEQUACY OF THE STUDY

This study is scientifically sound but is classified as **Supplemental** because of potential solvent effects on food consumption, which may have masked treatment-related effects of the test material. The results can be used quantitatively.

9. GUIDELINE DEVIATIONS

Although there are no specific U.S. EPA OCSPP guidelines available for this test, the reviewer assessed the study in accordance OECD Guideline No. 245 (2017).

There were two deviations from the guideline followed and one study deficiency:

1. Random or impartial assignment of bees to test groups was not reported.
2. The lighting condition of the study was not reported.
3. Potential solvent effects on food consumption (statistically significant reduction of food consumption in the solvent control compared to the negative control).

The potential solvent effects impact the acceptability of the study.

10. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Honey Bee (<i>Apis mellifera</i> L.)	Honeybee, <i>Apis mellifera</i> L. (Hymenoptera, Apidae)
<u>Age at beginning of test</u> Worker bees of uniform age.	Young adult worker bees (newly hatched, 1 to 2 days old).

Source	Bees were obtained from test facility stock in Niefern-Öschelbronn, Germany.
Were bees from diseased-free colonies?	Yes, the bees were from hives that were adequately fed, healthy and as far as possible disease-free and queen-right.
Were bees kept in conditions conforming to proper cultural practices?	<p>Yes, the colonies were examined for reportable bee epidemics by an authorized bee specialist and were inspected periodically according to the standard bee-keeping practices by an experienced apiarist.</p> <p>No chemical substances (such as antibiotics, anti-<i>Varroa</i> treatments, pesticides, etc.) had been used in the hive for at least 1 month prior to this test.</p>
Acclimation conditions	<p>Up to two days prior to start of exposure, brood combs containing capped cells which were expected to hatch on the same day were taken out of a honey bee colony and transferred into the climatic chamber. One day prior to start of exposure the ≤ 1-day old bees were picked off the combs, transferred to the test cages and kept under test conditions until start of exposure.</p> <p>50% (w/v) aqueous sucrose solution was used as food <i>ad libitum</i> during acclimation; the temperature ranged from 31.7 to 33.3 °C, and the relative humidity ranged from 39.4[*] to 63.4%.</p> <p>[*] The study author marked the lower humidity range value as part of short-term deviation values, implying that all values were within the recommended OECD short term deviation of ≤ 2 hours per day.</p>

B. Test System

Guideline Criteria	Reported Information
Plot size	N/A
Test Chambers	Stainless steel cages (base: 8 cm x 4 cm, height: 6 cm). The front side of each cage was equipped with a transparent pane to enable observation. The bottom of each cage consisted of perforated steel for sufficient air supply and was lined with filter paper.
Temperature during exposure	31.3 to 33.4 °C
Relative humidity during exposure	39.6* to 66.1% * The study author marked the lower humidity range value as part of short-term deviation values, implying that all values were within the recommended OECD short term deviation of ≤ 2 hours per day.
Lighting	Not reported
Feeding	50% (w/v) aqueous sucrose solution containing 5% acetone and 0.1% Xanthan offered <i>ad libitum</i> via syringes.

C. Test Design

Guideline Criteria	Reported Information
Maximum proposed label rate	Not reported
<u>Test material</u>	Identity: Mesotrione Technical Synonym: Mesotrione wet paste IUPAC name: 2-(4-mesyl-2-nitrobenzoyl)cyclohexane-1,3-dione CAS No.: 104206-82-8 Batch No: SMO0H028 Description: Brown solid Purity a.i.: (analyzed) 84.6% w/w Storage: Ambient (5 °C - 30 °C), dark, dry
<u>Nominal application rates</u> The test material should be applied at the maximum proposed label rate.	<u>Diet Concentrations:</u> 62.5, 125, 250, 500, and 1000 mg ai/kg diet <u>Actual Intake Dietary Doses:</u> 1.41, 2.73, 5.92, 13.30, and 21.85 µg ai/bee/day
Dose Preparation	A primary test item stock solution was prepared with 703 mg mesotrione filled to a volume 25 mL with acetone. This primary stock solution was serially diluted with acetone to prepare four additional secondary stock solutions. 2.5 mL of the primary stock solution was filled up to a final volume of 50 mL with 50% (w/v) aqueous sucrose solution containing 0.1% Xanthan to prepare the highest concentration feeding solution. The remaining feeding solutions were prepared in a similar manner with the additional secondary stock solutions.

Guideline Criteria	Reported Information
Number of bees exposed	<p>4 replicates each in a negative control, solvent control and five treatment groups.</p> <p>Each replicate contained 10 bees, for a total of 40 bees per control and treatment level.</p>
Application methods	<p>A volume of about 3-4 mL of feeding solution was offered to the bees, <i>ad libitum</i>, each 24 hour feeding interval via plastic syringes.</p> <p>The bees in one cage shared the feeding solution and thus received similar doses (trophallaxis).</p> <p>Freshly prepared feeding solution replaced the feeding solution of the previous day by changed feeders. The amount of feeding solution(s) consumed was determined by weighing the feeders before and after feeding using calibrated equipment.</p>

Guideline Criteria	Reported Information
Other experimental design information	<p>All 50% sucrose solutions were prepared with deionized water and stored, refrigerated, for up to 4 days.</p> <p>Feeders were weighed before and after they were offered to determine the mean food consumption of the bees per replicate. The individual daily consumption was corrected each day by the number of surviving bees at each assessment date as well as by estimated sucrose evaporation. Estimates of evaporation were determined during the study by maintaining syringes of 8 additional cages without bees under identical test conditions.</p> <p>Mortality, behavioral abnormalities, and food consumption was assessed daily throughout the 10-day exposure.</p> <p>Analytical samples and retain samples of the solvent control, all test item treatment groups, pure solvent (acetone) and the stock solution were taken every day directly after preparation. The concentration of Mesotrione was analyzed in the samples of the test item treated feeding solutions of each test item group and the solvent control group by HPLC-MS/MS.</p>
Were bees randomly or impartially assigned to test groups?	Not reported
Controls	<p>Negative control: 50% (w/v) untreated aqueous sucrose solution.</p> <p>Solvent control: 50% (w/v) aqueous sucrose solution containing 5 % Acetone and 0.1% Xanthan.</p>

Guideline Criteria	Reported Information
<u>Exposure period</u> 24 hours	10 days
<u>Positive Control</u>	50% (w/v) aqueous sucrose solution containing one concentration of Dimethoate, tested at a nominal concentration of 0.9 mg ai/kg diet.

11. **REPORTED RESULTS**

Guideline Criteria	Reported Information
Quality assurance, GLP compliance, No Claim of Confidentiality statements were included in the report?	Yes. This study was conducted in accordance with Organisation for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (GLP) and Compliance Monitoring (as revised in 1997) ENV/MC/CHEM (98)17 with one exception noted: 1) Inspections of honey bee colonies according to the standard bee keeping practices and stock keeping of honey bees were not conducted under GLP.
Control mortality	5% negative control mortality 5% solvent control mortality
Were raw data included?	Yes
Were signs of toxicity described?	No remarkable behavioral abnormalities were observed in any of the control or treatment groups.

Mortality and Observations

Measured Actual Intake Dietary Dose ($\mu\text{g ai/bee/day}$) ^a		Number Exposed	Mortality at 10 days (%) ^b	Behavioral Abnormalities
Negative Control		40	5	None
Solvent Control		40	5	None
Mesotrione	1.52	40	15	None
	2.5	40	0	None
	5.3	40	3	None
	12	40	3	None
	19	40	0	None
Positive Control, Dimethoate ^c	0.9 mg ai/kg diet	40	100	Not reported

^a Calculated by the reviewer; equivalent to mean measured concentrations of 67.7, 115, 224, 443, and 880 mg ai/kg diet.

^b Data obtained from Table 7 on p. 34 of the MRID.

^c The positive control caused 100% mortality by Day 7

After 10 days, mortality was 5% in both the negative and solvent controls compared to mortality ranging from 0 to 15% in the treatment groups. Mean food consumption over the course of the study was 29.6 and 22.0 mg/bee/day in the negative and solvent controls, respectively, compared to mean consumption of 22.6, 21.9, 23.7, 26.6, and 21.8 mg/bee/day in the measured actual intake dietary dose groups of 1.52, 2.5, 5.3, 12, and 19 $\mu\text{g ai/bee/day}$, respectively. No behavioral abnormalities were observed in any of the control or treatment groups throughout the 10-day test period.

Reported Statistics

A Chi² test with a Bonferroni-Correction (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there were significant differences between the mortality data of the solvent control and the test item treatment group and to determine the NOAEC and NOAEL based on mortality, respectively.

The LC/LD₅₀ after 10 days of continuous exposure could not be determined due to the lack of mortality above 50% and was estimated.

Statistical calculations were made by using the statistical program ToxRat Professional 3.2.1.

Based on nominal concentrations, the 10-day LC_{50} reported by the study author was >1000 mg ai/kg diet and the $NOAEC = 1000$ mg ai/kg diet. The 10-day LD_{50} reported by the study author was >21.9 μ g ai/bee/day and the $NOAEL = 21.9$ μ g ai/bee/day.

12. VERIFICATION OF STATISTICAL RESULTS

Mortality and food consumption data were analyzed using CETIS statistical software version 1.9.5.3 with database backend settings implemented by EFED on 7/25/17. Mean-measured diet concentrations calculated by the study author and measured actual intake dietary doses calculated by the reviewer were used for analysis and reporting and are represented in CETIS as separate records.

Negative and solvent control data were compared using an Equal Variance t Two-Sample test ($\alpha = 0.05$). A significant difference was determined for food consumption. All subsequent hypothesis testing was conducted comparing treatment data to negative control data only.

A Dunnett Multiple Comparison test ($\alpha = 0.05$) was used to compare treatment data for both endpoints to negative control data, as assumptions of normality and homoscedasticity were met based on a Shapiro-Wilk W Normality test ($\alpha = 0.01$) and a Mod Levene Equality of Variance or Bartlett Equality of Variance test ($\alpha = 0.01$), respectively.

For mortality, due to a lack of significant effects and lack of a dose response, the LC/LD_{50} values were empirically estimated as greater than the highest concentration/dose tested. Nonlinear regression was used in an attempt estimate IC/ID_{50} values for food consumption, but the analysis would not yield valid estimates or confidence intervals due to a lack of a dose response.

	Mortality	Food Consumption
Dietary Concentration (mg ai/kg diet)	$LC_{50} > 880$ 95% C.I.: N/A Slope: N/A $NOAEC \geq 880$ $LOAEC > 880$	$IC_{50} > 880$ 95% C.I.: N/A Slope: N/A $NOAEC < 67.7$ $LOAEC \leq 67.7$
Dietary Dose (μ g ai/bee/day)	$LD_{50} > 19$ 95% C.I.: N/A Slope: N/A $NOAEL \geq 19$ $LOAEL > 19$	$ID_{50} > 19$ 95% C.I.: N/A Slope: N/A $NOAEL < 1.52$ $LOAEL \leq 1.52$

13. REVIEWER'S COMMENTS

The reviewer's and the study author's results were in agreement over the LC/LD₅₀ and level that constituted the NOAEC/LOAEC when accounting for differences between nominal concentrations and doses (used by the study author) and measured concentrations and doses (used by the reviewer) for analysis and reporting. The study author did not statistically evaluate food consumption whereas the reviewer did. The reviewer's results are presented in the Conclusions section of this DER.

The study author evaluated the chronic effects of mesotrione by comparing the results of the test item group to those of the solvent control group. The reviewers compared results to the negative control group.

There was a statistically significant difference between the negative control and all but one of the treatment groups based on reduced food consumption, resulting in a NOAEC/L less than the lowest treatment-level group. However, the amount of food consumption in the treatment groups was consistent with that in the solvent control, which also showed a statistically significant reduction in food consumption compared to the negative control. Therefore, this study does not demonstrate any treatment-related effects on food consumption. Nonetheless, the potential solvent effect may have masked any treatment-related effects that may have occurred.

The OECD Acceptability Criteria for the guideline used was met in this study.

The in-life phase of this test was conducted from July 9 to August 22, 2018.

14. REFERENCES

Abbott, W.S. (1925): A method of computing the effectiveness of an insecticide. Jour. Econ. Entomol., 18, 265-267.

Hoelle, J. (2018) : Mesotrione - Analytical Method and Validation ECO_047_03A for the Determination of Mesotrione in Sucrose Feeding Solutions from Honey Bee Ecotoxicology Studies. Eurofins Agroscience Services EcoChem GmbH, Eutinger Str. 24, D-75223 Niefern-Öschelbronn, Germany. Unpublished report No S18 03364.

Schneider-Orelli, O. (1947): Entomologisches Praktikum. H.R. Sauerländer & Co., Aarau, 2. Auflage; [Schneider-Orelli, O. (1947): Entomological Practical Course. H.R. Sauerländer & Co., Aarau, 2nd Edition].

ToxRat Solutions GmbH, ToxRat Professional 3.2.1.

All other references were standard guidelines or methodologies.

CETIS Summary Report

Report Date: 03 Apr-19 12:46 (p 1 of 2)
Test Code/ID: 50743201 dd / 08-3728-9957

Special Study Honey bee Adult Chronic Oral Toxicity, 10-day Study

Eurofins Agrosience Service GmbH

Batch ID: 02-4697-2698	Test Type: 2014 Honeybee Adult Chron Oral	Analyst:
Start Date: 09 Jul-18	Protocol: Honeybee Adult Chronic Oral Toxicity, 10-d	Diluent:
Ending Date: 22 Aug-18	Species: Apis mellifera	Brine:
Test Length: 44d 0h	Taxon:	Source: Lab In-House Culture Age:
Sample ID: 01-3242-0293	Code: 50743201 dd	Project: Herbicide
Sample Date: 09 Jul-18	Material: Mesotrione	Source: Syngenta
Receipt Date:	CAS (PC):	Station:
Sample Age: n/a	Client: CDM Smith - M. Wright	

122990 50743201 - measured actual intake daily dietary doses

Single Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
14-5254-1750	Food Consumption	Equal Variance t Two-Sample Test	0.0297	Solvent Blank failed food consumption	1

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	TU	PMSD	S
09-3123-8411	10-Day Mortality Rate	Jonckheere-Terpstra Step-Down Test	19	>19	n/a		n/a	1
14-6139-5035	10-Day Mortality Rate	Mann-Whitney U Two-Sample Test	19	>19	n/a		15.1%	1
05-0789-1406	Food Consumption	Dunnett Multiple Comparison Test	<1.52	1.52	n/a		15.9%	1
11-8000-0914	Food Consumption	Williams Multiple Comparison Test	<1.52	1.52	n/a		12.3%	1

10-Day Mortality Rate Summary

Conc-µg ai/bee	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	4	0.050	0.000	0.142	0.000	0.100	0.029	0.058	115.47%	0.00%
0	N	4	0.050	0.000	0.142	0.000	0.100	0.029	0.058	115.47%	0.00%
1.52		4	0.150	0.000	0.529	0.000	0.500	0.119	0.238	158.70%	10.53%
2.5		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		-5.26%
5.3		4	0.025	0.000	0.105	0.000	0.100	0.025	0.050	200.00%	-2.63%
12		4	0.025	0.000	0.105	0.000	0.100	0.025	0.050	200.00%	-2.63%
19		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		-5.26%

Food Consumption Summary

Conc-µg ai/bee	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	S	4	22	17.7	26.3	18.8	25	1.35	2.7	12.27%	0.00%
0	N	4	29.7	22.3	37	25.1	33.9	2.32	4.65	15.67%	-34.62%
1.52		4	22.6	20.7	24.5	21	23.7	0.612	1.22	5.42%	-2.61%
2.5		4	21.9	20.1	23.6	20.6	23.2	0.556	1.11	5.09%	0.79%
5.3		4	23.7	19.3	28.1	20.9	27.4	1.38	2.76	11.63%	-7.60%
12		4	26.6	23.8	29.4	24.8	28.6	0.878	1.76	6.60%	-20.77%
19		4	21.9	16.6	27.1	17.7	25.8	1.66	3.31	15.14%	0.68%

CETIS Summary Report

Report Date: 03 Apr-19 12:46 (p 2 of 2)
Test Code/ID: 50743201 dd / 08-3728-9957

Special Study Honey bee Adult Chronic Oral Toxicity, 10-day Study

Eurofins Agrosience Service GmbH

10-Day Mortality Rate Detail

Conc-µg ai/bee	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	S	0.000	0.100	0.100	0.000
0	N	0.100	0.100	0.000	0.000
1.52		0.000	0.100	0.500	0.000
2.5		0.000	0.000	0.000	0.000
5.3		0.100	0.000	0.000	0.000
12		0.000	0.100	0.000	0.000
19		0.000	0.000	0.000	0.000

Food Consumption Detail

Conc-µg ai/bee	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	S	21	18.8	25	23.3
0	N	33.9	33.4	26.2	25.1
1.52		23.4	21	22.3	23.7
2.5		20.6	23.2	21.4	22.2
5.3		27.4	20.9	23.9	22.6
12		25.5	27.5	28.6	24.8
19		25.8	21.9	17.7	22.1

CETIS Analytical Report

Report Date: 03 Apr-19 12:46 (p 1 of 5)
Test Code/ID: 50743201 dd / 08-3728-9957

Special Study Honey bee Adult Chronic Oral Toxicity, 10-day Study

Eurofins Agrosience Service GmbH

Analysis ID: 14-6139-5035 Endpoint: 10-Day Mortality Rate CETIS Version: CETISv1.9.5
Analyzed: 03 Apr-19 12:43 Analysis: Nonparametric-Two Sample Status Level: 1

Batch ID: 02-4697-2698 Test Type: 2014 Honeybee Adult Chron Oral Analyst:
Start Date: 09 Jul-18 Protocol: Honeybee Adult Chronic Oral Toxicity, 10-d Diluent:
Ending Date: 22 Aug-18 Species: Apis mellifera Brine:
Test Length: 44d 0h Taxon: Source: Lab In-House Culture Age:

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C < T	19	>19	n/a		15.05%

Mann-Whitney U Two-Sample Test

Control	vs	Conc-µg ai/b	Test Stat	Critical	Ties	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		1.52	9	n/a	3	6	Exact	0.5000	Non-Significant Effect
		2.5	4	n/a	1	6	Exact	1.0000	Non-Significant Effect
		5.3	6	n/a	3	6	Exact	0.9286	Non-Significant Effect
		12	6	n/a	3	6	Exact	0.9286	Non-Significant Effect
		19	4	n/a	1	6	Exact	1.0000	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0633333	0.0126667	5	1.17	0.3621	Non-Significant Effect
Error	0.195	0.0108333	18			
Total	0.258333		23			

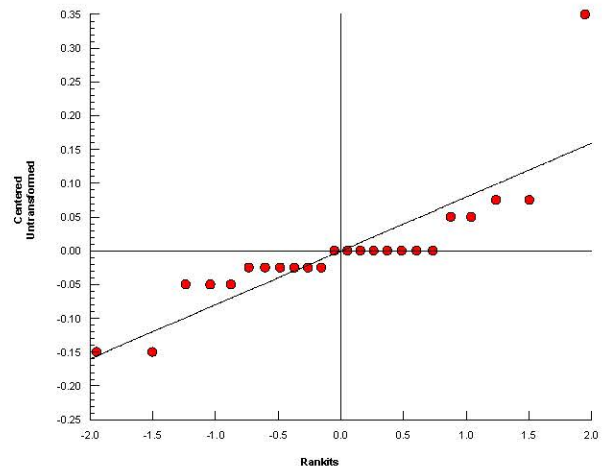
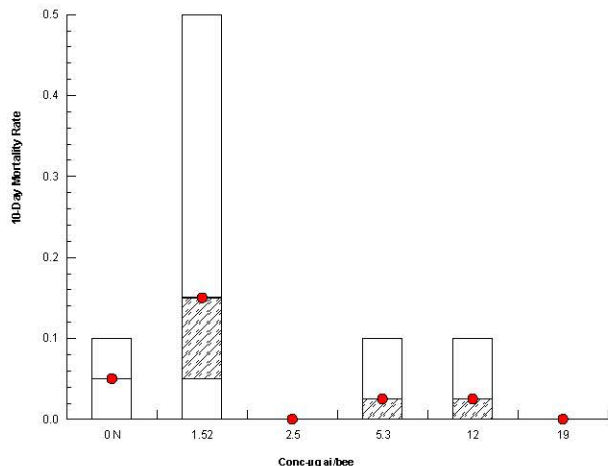
ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	5.88	4.25	0.0022	Unequal Variances
	Mod Levene Equality of Variance Test	1.69	4.25	0.1883	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.737	0.884	3.2E-05	Non-Normal Distribution

10-Day Mortality Rate Summary

Conc-µg ai/bee	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.050	0.000	0.142	0.050	0.000	0.100	0.029	115.47%	0.00%
1.52		4	0.150	0.000	0.529	0.050	0.000	0.500	0.119	158.70%	10.53%
2.5		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		-5.26%
5.3		4	0.025	0.000	0.105	0.000	0.000	0.100	0.025	200.00%	-2.63%
12		4	0.025	0.000	0.105	0.000	0.000	0.100	0.025	200.00%	-2.63%
19		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		-5.26%

Graphics



CETIS Analytical Report

Report Date: 03 Apr-19 12:46 (p 2 of 5)
Test Code/ID: 50743201 dd / 08-3728-9957

Special Study Honey bee Adult Chronic Oral Toxicity, 10-day Study

Eurofins Agrosience Service GmbH

Analysis ID: 09-3123-8411	Endpoint: 10-Day Mortality Rate	CETIS Version: CETISv1.9.5
Analyzed: 03 Apr-19 12:43	Analysis: Nonparametric-Control vs Ord. Treatments	Status Level: 1
Batch ID: 02-4697-2698	Test Type: 2014 Honeybee Adult Chron Oral	Analyst:
Start Date: 09 Jul-18	Protocol: Honeybee Adult Chronic Oral Toxicity, 10-d	Diluent:
Ending Date: 22 Aug-18	Species: Apis mellifera	Brine:
Test Length: 44d 0h	Taxon:	Source: Lab In-House Culture Age:

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU
Untransformed	C < T	19	>19	n/a	

Jonckheere-Terpstra Step-Down Test

Control	vs	Conc-µg ai/b	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Negative Control		1.52	0.316	1.64	2	Asymp	0.9464	Non-Significant Effect
		2.5	-1.24	1.64	2	Asymp	0.9464	Non-Significant Effect
		5.3	-1.1	1.64	2	Asymp	0.9464	Non-Significant Effect
		12	-0.999	1.64	2	Asymp	0.9464	Non-Significant Effect
		19	-1.61	1.64	2	Asymp	0.9464	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0633333	0.0126667	5	1.17	0.3621	Non-Significant Effect
Error	0.195	0.0108333	18			
Total	0.258333		23			

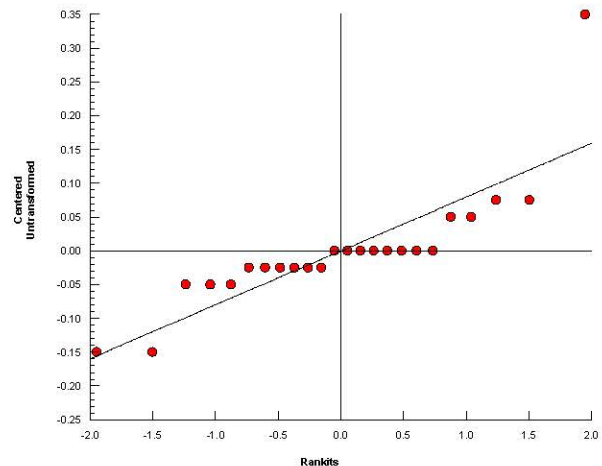
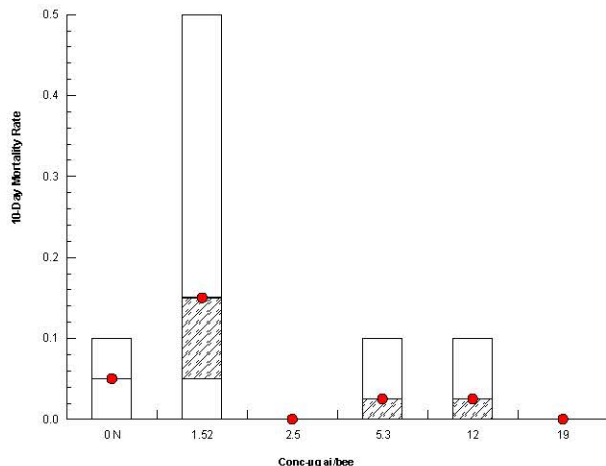
ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	5.88	4.25	0.0022	Unequal Variances
	Mod Levene Equality of Variance Test	1.69	4.25	0.1883	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.737	0.884	3.2E-05	Non-Normal Distribution

10-Day Mortality Rate Summary

Conc-µg ai/bee	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	4	0.050	0.000	0.142	0.050	0.000	0.100	0.029	115.47%	0.00%
1.52		4	0.150	0.000	0.529	0.050	0.000	0.500	0.119	158.70%	10.53%
2.5		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		-5.26%
5.3		4	0.025	0.000	0.105	0.000	0.000	0.100	0.025	200.00%	-2.63%
12		4	0.025	0.000	0.105	0.000	0.000	0.100	0.025	200.00%	-2.63%
19		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000		-5.26%

Graphics



CETIS Analytical Report

Report Date: 03 Apr-19 12:46 (p 3 of 5)
Test Code/ID: 50743201 dd / 08-3728-9957

Special Study Honey bee Adult Chronic Oral Toxicity, 10-day Study

Eurofins Agrosience Service GmbH

Analysis ID: 05-0789-1406 Endpoint: Food Consumption CETIS Version: CETISv1.9.5
Analyzed: 03 Apr-19 12:43 Analysis: Parametric-Control vs Treatments Status Level: 1

Batch ID: 02-4697-2698 Test Type: 2014 Honeybee Adult Chron Oral Analyst:
Start Date: 09 Jul-18 Protocol: Honeybee Adult Chronic Oral Toxicity, 10-d Diluent:
Ending Date: 22 Aug-18 Species: Apis mellifera Brine:
Test Length: 44d 0h Taxon: Source: Lab In-House Culture Age:

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	<1.52	1.52	n/a		15.89%

Dunnett Multiple Comparison Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		1.52*	3.6	2.41	4.71	6	CDF	0.0043	Significant Effect
		2.5*	3.99	2.41	4.71	6	CDF	0.0019	Significant Effect
		5.3*	3.04	2.41	4.71	6	CDF	0.0142	Significant Effect
		12	1.56	2.41	4.71	6	CDF	0.2097	Non-Significant Effect
		19*	3.97	2.41	4.71	6	CDF	0.0019	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	196.032	39.2064	5	5.12	0.0043	Significant Effect
Error	137.868	7.65931	18			
Total	333.9		23			

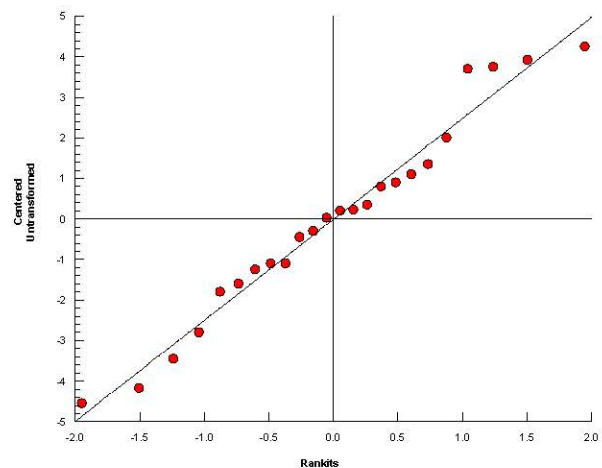
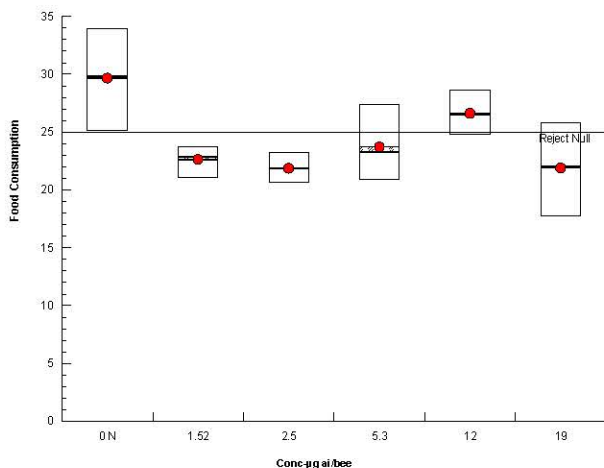
ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	7.91	15.1	0.1615	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.965	0.884	0.5447	Normal Distribution

Food Consumption Summary

Conc-µg ai/bee	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	% Effect
0	N	4	29.7	22.3	37	29.8	25.1	33.9	2.32	15.67%	0.00%
1.52		4	22.6	20.7	24.5	22.8	21	23.7	0.612	5.42%	23.78%
2.5		4	21.9	20.1	23.6	21.8	20.6	23.2	0.556	5.09%	26.31%
5.3		4	23.7	19.3	28.1	23.2	20.9	27.4	1.38	11.63%	20.07%
12		4	26.6	23.8	29.4	26.5	24.8	28.6	0.878	6.60%	10.29%
19		4	21.9	16.6	27.1	22	17.7	25.8	1.66	15.14%	26.22%

Graphics



CETIS Analytical Report

Report Date: 03 Apr-19 12:46 (p 4 of 5)
Test Code/ID: 50743201 dd / 08-3728-9957

Special Study Honey bee Adult Chronic Oral Toxicity, 10-day Study

Eurofins Agrosience Service GmbH

Analysis ID: 11-8000-0914	Endpoint: Food Consumption	CETIS Version: CETISv1.9.5
Analyzed: 03 Apr-19 12:44	Analysis: Parametric-Control vs Ord.Treatments	Status Level: 1
Batch ID: 02-4697-2698	Test Type: 2014 Honeybee Adult Chron Oral	Analyst:
Start Date: 09 Jul-18	Protocol: Honeybee Adult Chronic Oral Toxicity, 10-d	Diluent:
Ending Date: 22 Aug-18	Species: Apis mellifera	Brine:
Test Length: 44d 0h	Taxon:	Source: Lab In-House Culture Age:

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	<1.52	1.52	n/a		12.32%

Williams Multiple Comparison Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		1.52*	3.6	1.73	3.39	6	CDF	<0.05	Significant Effect
		2.5*	3.99	1.82	3.56	6	CDF	<0.05	Significant Effect
		5.3*	3.54	1.85	3.61	6	CDF	<0.05	Significant Effect
		12*	3.05	1.86	3.64	6	CDF	<0.05	Significant Effect
		19*	3.97	1.87	3.65	6	CDF	<0.05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	196.032	39.2064	5	5.12	0.0043	Significant Effect
Error	137.868	7.65931	18			
Total	333.9		23			

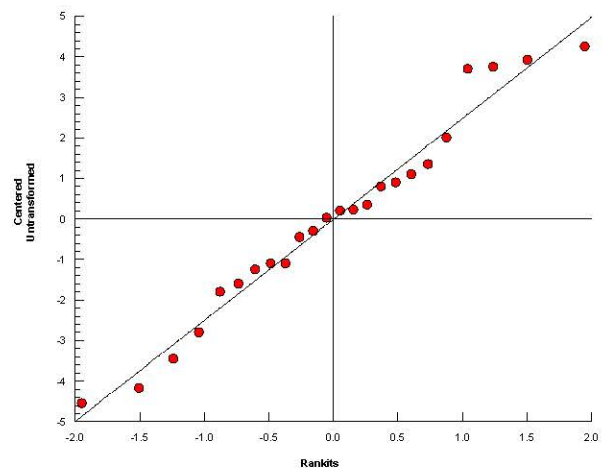
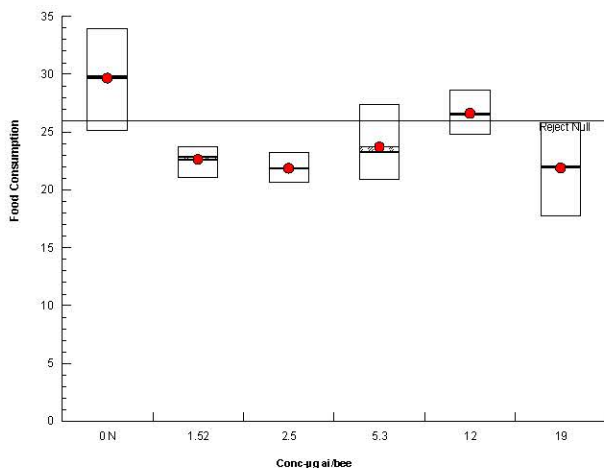
ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	7.91	15.1	0.1615	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.965	0.884	0.5447	Normal Distribution

Food Consumption Summary

Conc-µg ai/bee	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	% Effect
0	N	4	29.7	22.3	37	29.8	25.1	33.9	2.32	15.67%	0.00%
1.52		4	22.6	20.7	24.5	22.8	21	23.7	0.612	5.42%	23.78%
2.5		4	21.9	20.1	23.6	21.8	20.6	23.2	0.556	5.09%	26.31%
5.3		4	23.7	19.3	28.1	23.2	20.9	27.4	1.38	11.63%	20.07%
12		4	26.6	23.8	29.4	26.5	24.8	28.6	0.878	6.60%	10.29%
19		4	21.9	16.6	27.1	22	17.7	25.8	1.66	15.14%	26.22%

Graphics



CETIS Analytical Report

Report Date: 03 Apr-19 12:46 (p 5 of 5)
Test Code/ID: 50743201 dd / 08-3728-9957

Special Study Honey bee Adult Chronic Oral Toxicity, 10-day Study

Eurofins Agroscience Service GmbH

Analysis ID: 14-5254-1750	Endpoint: Food Consumption	CETIS Version: CETISv1.9.5
Analyzed: 03 Apr-19 12:44	Analysis: Parametric-Two Sample	Status Level: 1
Batch ID: 02-4697-2698	Test Type: 2014 Honeybee Adult Chron Oral	Analyst:
Start Date: 09 Jul-18	Protocol: Honeybee Adult Chronic Oral Toxicity, 10-d	Diluent:
Ending Date: 22 Aug-18	Species: Apis mellifera	Brine:
Test Length: 44d 0h	Taxon:	Source: Lab In-House Culture Age:

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C <> T	Solvent Blank failed food consumption	22.18%

Equal Variance t Two-Sample Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		Solvent Blank*	2.84	2.45	6.58	6	CDF	0.0297	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	116.281	116.281	1	8.05	0.0297	Significant Effect
Error	86.6575	14.4429	6			
Total	202.939		7			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Variance Ratio F Test	2.95	47.5	0.3977	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.897	0.645	0.2725	Normal Distribution

Food Consumption Summary

Conc-µg ai/bee	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	S	4	22	17.7	26.3	22.1	18.8	25	1.35	12.27%	0.00%
0	N	4	29.7	22.3	37	29.8	25.1	33.9	2.32	15.67%	-34.62%

Graphics

